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# Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation

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*The soil–lettuce lead transfer from atmospheric industrial sub-micronic and micronic particles depends on particle size.*

## A B S T R A C T

Particles from channelled emissions of a battery recycling facility were size-segregated and investigated to correlate their speciation and morphology with their transfer towards lettuce. Microculture experiments carried out with various calcareous soils spiked with micronic and sub-micronic particles ( $1650 \pm 20 \text{ mg Pb kg}^{-1}$ ) highlighted a greater transfer in soils mixed with the finest particles. According to XRD and Raman spectroscopy results, the two fractions presented differences in the amount of minor lead compounds like carbonates, but their speciation was quite similar, in decreasing order of abundance: PbS, PbSO<sub>4</sub>, PbSO<sub>4</sub>·PbO,  $\alpha$ -PbO and Pb<sup>0</sup>. Morphology investigations revealed that PM<sub>2.5</sub> (i.e. Particulate Matter 2.5 composed of particles suspended in air with aerodynamic diameters of 2.5  $\mu\text{m}$  or less) contained many Pb nanoballs and nanocrystals which could influence lead availability. The soil–plant transfer of lead was mainly influenced by size and was very well estimated by 0.01 M CaCl<sub>2</sub> extraction.

## Keywords:

Lead  
PM<sub>2.5</sub> and PM<sub>10</sub>  
Soil–lettuce transfer  
Phytoavailability

## 1. Introduction

Due to its numerous past and present uses and high persistence, lead is a major environmental contaminant (Chen et al., 2005). Potentially toxic for living organisms even at low concentrations, lead constitutes a risk for humans who can absorb it in various ways (Canfield et al., 2003). In the context of contaminated gardens, elevated lead intake by humans can be due to the consumption of crop plants grown on soils with relatively high plant-available metal concentrations, ingestion of contaminated soil, either accidentally or intentionally (pica), inhalation of soil particles and drinking water with high soluble concentrations of metals (Alexander et al., 2006). The total quantities of lead emitted in the environment by industries have decreased sharply in recent decades (Glorennec et al., 2007) and are strictly controlled in Europe nowadays. Lead was recently classified as a substance of very high concern in the European REACH law (Regulation EC 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals). However, particles enriched with lead are

still generated especially by lead-recycling plants (Batonneau et al., 2004; Ohmsen, 2001) and constitute the main source of lead pollution for soils (Miquel, 2001; Donisa et al., 2000).

According to Zhang et al. (2005), emitted particles present a large granulometric spectrum in the atmosphere, but during the last decade the proportion of fine particle matter (PM) increased with the use of more effective filters in industry. Indeed PM<sub>10</sub> are target species of the World Health Organization (WHO, 1987) and the European Union Framework Directive on ambient air quality assessment (European Commission, 1999), due to their adverse effects on the environment and human health. While micrometric and sub-micrometric fractions contribute very little to ambient particle mass, they may occur in substantial number concentrations. Most of the studies dealing with the characterization of metal-enriched particles in the ambient air provide information on quantitative measurements for PM<sub>10</sub> fractions (EU directives 96/62 and 99/30) and very few on the sub-micronic fraction (Lazaridis et al., 2002). The lack of knowledge regarding metal speciation in the industrial particles results mainly from a lack of analytical tools, both sensitive and specific to the size of the particles.

These fine particles are highly reactive due to their high specific area and can be transported over long distances in the troposphere (Barrie, 1992). They could therefore present a greater impact on the biosphere than coarse particles (Fernandez Espinosa and Rossini Oliva, 2006). Ruby et al. (1992) concluded that the bioaccessibility of

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lead rises strongly in particles under 2.4  $\mu\text{m}$  size. But, the phytoavailability of lead in industrial particles as a function of their size and speciation has not been studied yet. In comparison with zinc, lead generally shows a relatively low mobility in soils (Dumat et al., 2006). It can however migrate through the soil with dissolved organic matter (Cecchi et al., 2008) or be mobilized by certain plants (Arshad et al., 2008). Moreover, carried from the air to the soils as fine particles, lead could be released more easily in soil solution (Komarnicki, 2005).

We therefore focused our study on the links between soil–plant transfer of lead, size and speciation of particles emitted by a lead-recycling plant, currently the main source of atmospheric emissions (Cecchi et al., 2008). The objectives were the following: (i) the elemental and molecular characterization of micrometer and nanometer sized lead-rich particles and (ii) to study the influence of particle characteristics on lead soil–plant transfer.

The physico-chemical characterization of industrial  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  particles collected in the plant was investigated using both bulk and microanalysis techniques: (i) MEB-EDS to determine the morphology and chemistry on the scale of a particle (Laskin et al., 2006; Choël et al., 2005, 2006); (ii) Raman microspectrometry to study particle speciation (Batonneau et al., 2004, 2006; Falgayrac et al., 2006; Sobanska et al., 2006). The transfer of lead from particles to the lettuce, *Lactuca sativa*, a widely grown garden vegetable was investigated in the laboratory: two different uncontaminated calcareous soils were spiked with  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  for soil–plant experiments with a biotest device that enabled careful study of rhizosphere and roots in addition to the transfer to the shoots (Chaignon and Hinsinger, 2003). The study was finally completed by  $\text{CaCl}_2$  extraction experiments carried out according to Houba et al. (1996) to estimate lead phytoavailability.

The hypothesis tested throughout all these experiments was that particle characteristics have a significant influence on lead soil–plant transfer and translocation.

## 2. Materials and methods

### 2.1. Particle sampling and size separation

A secondary lead smelter which currently recycles batteries was chosen as a representative example of the smelter metal industry to develop a methodology aimed at the risk assessment of industrial lead particles. The plant of the Chemical Metal Treatment Company (STCM) is located in the urban area of Toulouse (43°38'12" N, 01°25'34" E). According to the French authorities (DRIRE, 2007), 328 kg of Total Suspended Particles (TSP) including 31 kg of lead were emitted by this factory in 2007.

Three sources of particles corresponding to the three work units involving different steps in the process are identified in the plant: (i) the battery grinding unit where battery components are separated under wet conditions (ii) the smelter where lead pastes are processed in rotary furnaces at 1200 °C and finally (iii) the refinery where lead is purified from unwanted metals or enriched. The same process has been used for thirty years in the plant. This study focuses on channelled emissions only generated by the furnace unit, considering the transfer towards the biosphere. The particle collection, performed three months after complete cleaning of the three work units (March, 12, 2007), is therefore representative of that emission period. Our aim was to characterize the reactivity of the particles in relation with their previously determined speciation and size, not to follow one specific parameter over time.

1 kg of particles was collected in polyethylene bags from the air-sleeve filters of the furnace, then sealed and transferred in sealed opaque containers for transport to the laboratory. Samples were passed through a 2-mm stainless steel AFNOR sieve. Process dust was stored in a cool (4 °C) dark place with Merck desiccant.

Because 80% of emitted particles were smaller than 10  $\mu\text{m}$ ,  $\text{PM}_{2.5}$  and  $\text{PM}_{10}$  were size-segregated by artificial resuspension in a Teflon bag (Batonneau et al., 2004; Young et al., 2002) and collected by impactation onto a  $\text{PM}_{10/2.5}$  Dekati inertial impactor. The cascade impactor consists of two successive stages with aerodynamic cut-off diameters of 10 and 2.5  $\mu\text{m}$  when it operates at 10 L/min airflow.  $\text{PM}_{10}$  present an aerodynamic diameter between 2.5 and 10  $\mu\text{m}$ , whereas the  $\text{PM}_{2.5}$  stage collects particles <2.5  $\mu\text{m}$ .

### 2.2. Characterization of particles

Elemental total contents of the two size fractions were determined by ICP-OES (IRIS Intrepid II XDL) after heated digestion with standard acid ( $\text{HNO}_3$ ,  $\text{HCl}$  and  $\text{HF}$ ,

Suprapur, Merk) in a PTFE vessel. Levels of C, H, S and O were determined after burning in an elemental analyser with coulometric–catharometric detection and IR.

X-ray diffraction patterns of  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  were recorded on an INEL diffractometer equipped with a curved detector CPS 120 and Co ( $K\alpha$ ) radiation allowing a  $120^\circ/2\theta$  detection. Crystallized compounds were identified by comparison with the diffraction patterns of the JCPDS database. The relative abundance of each crystallized phase detected in samples was qualitatively estimated by using the relative intensity of the strongest X-ray pattern peaks of each considered phase. It should be noticed that only crystallized phases with an abundance superior to 5% in weight can be detected by XRD.

Particles were characterized using complementary microscopy and imaging techniques to determine elementary and molecular composition, size, morphology and heterogeneity of the individual lead-rich particles.

X-ray elemental mapping and secondary images were obtained using an Environmental Scanning Electron Microscope (ESEM) working in high-vacuum mode. The Quanta 200 FEI instrument was equipped with an energy-dispersive X-ray (EDX) detector. ESEM was operated at 10–25 kV. Particles were dispersed on carbon substrates and analysed without further preparation. Because of the ESEM configuration, light element detection (C, N, and O) was ambiguous.

Raman microspectrometric measurements were carried out with a LabRAM confocal spectrometer (Jobin Yvon, Horiba Gr, France). The spot size of the laser focused by a 100 $\times$  objective (numerical aperture (NA) = 0.90) was estimated to be 1  $\mu\text{m}^2$  in size. A liquid nitrogen-cooled CCD (Jobin Yvon, 2048 pixels  $\times$  512 pixels) was used for detection. The Raman backscattering was excited at 632.8 nm provided by an internal, air-cooled, linearly polarized helium–neon laser. The laser power delivered to the sample was 8 mW. The microscope stage was XY-motorized and computer-controlled for point-by-point scanning with 0.1  $\mu\text{m}$  resolution, 1  $\mu\text{m}$  reproducibility and 90 mm  $\times$  60 mm spatial range. The glass plate with the impacted particles was mounted on the microscope stage without any further preparation. Data acquisition consisted in recording many spectra in point-by-point scanning mode with 1  $\mu\text{m}$  as a minimum step, one accumulation and 30 s spectrum acquisition time. For identification of Raman spectra the experimental spectra were compared to reference spectra using Spectral Library Search ID 301 software (Thermo Galactic).

### 2.3. Measure of soil–plant lead transfer performing microculture experiments

Lettuce was chosen because it is a common vegetable widely grown for human consumption and has recently been used by several authors for metal transfer studies (Khan et al., 2008; Waisberg et al., 2004; Alexander et al., 2006). Moreover, lettuce is often used around plants to estimate atmospheric pollution.

The biotest presented in Fig. 1 was first described by Niebes et al. (1993) and then adapted by Chaignon and Hinsinger (2003). A small PVC cylinder (25 mm inner diameter) was closed by a polyamide net (900  $\mu\text{m}$  mesh) inserted into a larger cylinder, itself closed by a finer polyamide mesh (30  $\mu\text{m}$ , Fyltis/Nytel, Sefar filtration). A space of 3 mm was left between the net and the finer mesh, where the roots could develop as a mat.

Commercial lettuce seeds, “Batavia blonde dorée” cultivar, were surface sterilized with 0.9%  $\text{CaClO}$  for 15 min and rinsed with deionised water. Three seeds were sown in the container on the surface of the coarser mesh.

Lettuces were first grown hydroponically for 21 days to obtain a large flat mass of roots that fully covered the fine mesh.

The devices were placed on top of troughs containing an aerated complete nutrient solution with the macroelements: 5 mM  $\text{KNO}_3$ , 5 mM  $\text{Ca}(\text{NO}_3)_2$ , 2 mM  $\text{KH}_2\text{PO}_4$  and 1.5 mM  $\text{MgSO}_4$  and oligoelements: 9.11  $\mu\text{M}$   $\text{MnSO}_4$ , 1.53  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.235  $\mu\text{M}$   $\text{CuSO}_4$ , 24.05  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 0.1  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$  and 268.6  $\mu\text{M}$   $\text{Fe}/\text{EDTA}$ . The height of the nutrient solution was adjusted daily to keep the fine mesh wet during the whole pre-culture period. The experiment was conducted in a growth chamber (temperature  $24 \pm 0.5^\circ\text{C}/18 \pm 0.5^\circ\text{C}$  day/night cycles; photoperiod 16 h under daylight fluorescent lamps providing 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  [Philips 600 W, Eindhoven, Netherlands] and 8 h darkness; relative humidity 70%).

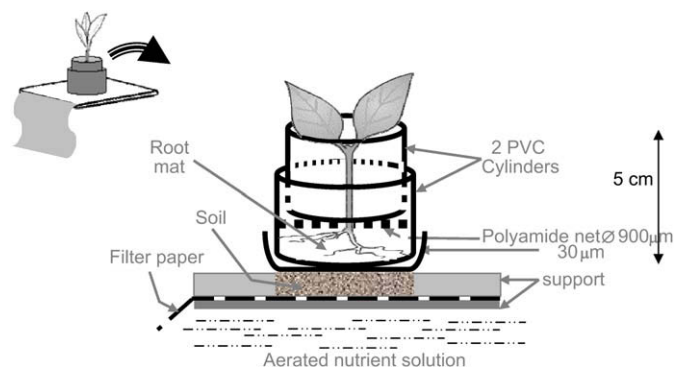


Fig. 1. Microculture device.

**Table 1**  
Physico-chemical characteristics of the two soils used for microculture experiments

	Unit	Soil-1	Soil-2
pH		8.3	8.4
CEC	cmol+/kg	19.9	8.9
Clay	(g kg <sup>-1</sup> )	405	195
Silt		261	390
Sand		334	415
C/N		9.15	8.9
MO	(g kg <sup>-1</sup> )	18.8	12.5
Total carbonates	(g kg <sup>-1</sup> )	36.8	98
(P <sub>2</sub> O <sub>5</sub> ) <sub>Forest Hebert</sub>	(g kg <sup>-1</sup> )	0.28	0.215
Cd (HF extraction)	(mg kg <sup>-1</sup> )	0.22	0.175
Pb (HF)		35.5	21
Zn (HF)		101	48
Cu (HF)		28.5	31

Then, the second step involved an eight-day soil-plant contact period: five replicate plants for each treatment were transferred onto the various soil samples.

Top soils of two calcic cambisol profiles (FAO, 1998) produced by quaternary alluvial deposits, were sampled. These two calcareous soils (noted soil-1 and soil-2) with various physico-chemical properties (Table 1) were chosen because that kind of soil happens to be observed around several battery recycling plants. Moreover, if the lettuce species could favor lead transfer, the choice of calcareous soils could reduce it. The soils differ by texture, soil cultivation, organic matter (OM) and CEC: soil-1 was uncultivated loamy-sandy clay with 2% of OM and soil-2 was sandy-clayey loam under sunflowers with 1% of OM and a higher CEC.

These two soils were spiked with PM<sub>2.5</sub> and PM<sub>10</sub> up to [Pb] = 1650 ± 20 mg kg<sup>-1</sup>. 60 mg of particles were added per box containing 10 g of soil and were agitated for a day. Soils were turned over every fifteen days for four months. This long period was chosen to allow time for natural equilibration of the various sorption mechanisms in the soil (Alexander et al., 2006). The relatively high total lead soil concentration was chosen to be representative of a real soil pollution situation observed for atmospheric fallout from an STCM plant (Cecchi et al., 2008) and we wanted to be sure that enough lead was available for the plant in soil solution. Surveys of garden soils in several countries have shown wide ranges of concentrations of heavy metals. For example, Culbard et al. (1988) found concentrations of up to 14,100 mg Pb kg<sup>-1</sup>. Some soils were left without crops, to determine the pH possible variation due to soil solution influence. During the growth, pH was measured daily and nutrient solution level was maintained constant. pH of nutrient solution was 5.5 ± 0.3 during the hydroponic and soil contact phases.

This biotest device presents two main advantages: (i) the roots are physically separated from the soil which enables total recovery of the shoots, roots and soil; (ii) the thickness of the soil layer used enables it to be considered it as rhizosphere soil and provides enough rhizosphere material to evaluate root-induced changes in metal speciation.

Roots and aerial parts were collected and analysed separately after the soil-plant contact period. Biomass was determined before oven-drying at 80 °C for 48 h. Lead bound to the outer root cell walls, called [Pb]<sub>adsorbed</sub>, was determined according to the method of acidic desorption as described by Ferrand et al. (2006): roots were shaken end over end with 40 mL of 0.001 M HCl for 3 min, and then 360 µL of 1 M HCl was added to yield a final concentration of 0.01 M HCl. After shaking for another 5 min, the suspension was filtered through ashless paper. Then, plant roots and aerial parts were mineralized separately in a 1:1 mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> at 80 °C for 4 h. After filtration, the major and trace element concentrations were determined with an IRIS Intrepid II XDL ICP-OES. The concentration in roots after mineralization is considered as uptaken lead ([Pb]<sub>roots</sub>). The accuracy of the acidic digestion and analytical procedures was verified using the reference material Virginia tobacco leaves, CTA-VTL-2, ICHTJ.

#### 2.4. Estimation of lead availability by CaCl<sub>2</sub> extraction experiments

For the determination of the phytoavailable fraction, according to Menzies et al. (2007) neutral salts extractants provide the most useful indication. The 0.01 M CaCl<sub>2</sub> extraction procedure gives a good indication of lead phytoavailability (Meers et al., 2007). The CaCl<sub>2</sub> procedure, first described by Houba et al. (1996), was performed on the two contaminated soils and particle samples (PM<sub>10</sub> and PM<sub>2.5</sub>). 30 mL of 0.01 M CaCl<sub>2</sub> solution was mixed with 3 g of soil (1:10 solid solution ratio) in 50 mL

polypropylene centrifugation tubes placed on a shaker table (Heidolph promax 1020) at 50 oscillations/min for 2 h, 20 °C. After extraction, the tubes were centrifuged at 10,000g for 30 min (Avanti 30 centrifuge Beckman). The supernatant liquid was then filtered (0.22 µm), acidified to 2% with distilled HNO<sub>3</sub> (15 M, suprapur 99.9%) and stored at 4 °C before analysis. That CaCl<sub>2</sub> extraction procedure was performed on particles, reference soils, and the two spiked soils before and after culture. Extracted metal concentrations were determined by an IRIS Intrepid II XDL/Thermo Electron Corporation model ICP-AES Calibration used reference materials (ion 915 from National Water Research Institute, Canada).

#### 2.5. Statistical data treatment

The plant absorption and chemical extraction data obtained were subjected to analysis of variance (ANOVA) with one factor, using the software Statistica, Edition'98 (StatSoft Inc., Tulsa, OK, USA). For each bioassay, mean values with different letters represent a significant difference (*p* < 0.05) as measured by the LSD Fisher test. Letters are reported on the figures and tables.

### 3. Results

#### 3.1. PM<sub>10</sub> and PM<sub>2.5</sub> characterization

Elemental concentrations in particles are shown in Table 2. All results are given as the mean of the three replicates for each sample (PM<sub>10</sub> and PM<sub>2.5</sub>) and standard deviations never exceed 7%. No significant difference except for Fe in the total elemental concentrations was observed in relation with the size of the particle. Major elements found in the samples were, by mass: Pb (27%), O (15%) and S (7.5%) for both fractions. High levels of Na (3–4%) were due to the industrial recycling process where Na is used to lower the melting point of Pb. Several other metals: Cd (2.5%), Zn (0.5%), Fe (0.1–0.4%) and Sb (0.1%) are also present. The remaining elements to complete 100% are expected to be chloride and other trace metals.

XRD patterns of PM<sub>10</sub> and PM<sub>2.5</sub> provide identification of major crystallized species in bulk samples. The results are presented in Table 3. Particles are mainly composed of metallic sulphides, sulphates, oxides and perchlorates. A significant amount of Na<sub>2</sub>SO<sub>4</sub> was found in PM<sub>10</sub> samples. Regardless of the size, the major phases identified were the same and one more Fe species was detected for PM<sub>2.5</sub>.

The ESEM-EDX analysis provided the morphology and elemental composition of individual particles in the two size fractions. PM<sub>10</sub> samples exhibited both particles with a size range between 2 and 10 µm and large aggregates composed of many micron-sized particles without specific shapes (Fig. 2). The main elements detected by EDX in these aggregates were, by order of importance, Pb, S, Cl, Sn, Na, and Fe. The chemical complexity of aggregates did not allow the detection of minor elements. Elemental mapping recorded on PM<sub>10</sub> showed the chemical heterogeneity of aggregates. PM<sub>2.5</sub> samples are composed of fine aggregates of few sub-micronic particles exhibiting characteristic features, i.e. needles (<100 nm), nanocrystals (<500 nm), cubes (~300 nm) and balls (~500 nm). Elemental analysis of particles showed that needles mainly contain Pb and S, nanocrystals are composed of Pb, S and Cl while Na and S are detected in cubic particles. Compared to the PM<sub>10</sub> sample, numerous Pb only-rich nanoballs were observed in PM<sub>2.5</sub> (Fig. 2).

Raman microspectrometry combines the analytical capability of Raman spectroscopy to distinguish *in situ* a wide range of chemical substances in aerosols with the spatial resolution of a confocal optical microscope (~1 µm<sup>3</sup>) which enables the investigation of

**Table 2**  
Total elemental analysis of particles

Sample	Al	As	Cd	Cu	Fe	O	Na	Ni	Pb	Sb	Zn	S	C
	Contents (mg kg <sup>-1</sup> ) ± 7%												
PM <sub>2.5</sub>	10	867	25,243	480	1395	149,300	31,691	41	272,834	1266	5194	78,100	12,100
PM <sub>10</sub>	52	841	23,139	617	4010	150,000	39,697	97	267,353	1361	5372	74,300	11,500

**Table 3**  
Determination of main chemical speciation of lead in the PM<sub>10</sub> and PM<sub>2.5</sub> particles by X-ray powder diffraction analysis

Sample	Crystallized compound	Estimated % of phases
PM <sub>10</sub>	<b>PbS</b>	55
	<b>PbO·PbSO<sub>4</sub></b> , <b>PbSO<sub>4</sub></b> , Pb(ClO <sub>4</sub> ) <sub>2</sub> , Pb	25
	<b>Na<sub>2</sub>SO<sub>4</sub></b>	10
	CdS, Cd(ClO <sub>4</sub> ) <sub>2</sub> , ZnO, ZnSO <sub>4</sub>	10
PM <sub>2.5</sub>	<b>PbS</b>	55
	<b>PbO·PbSO<sub>4</sub></b> , <b>PbSO<sub>4</sub></b> , Pb(ClO <sub>4</sub> ) <sub>2</sub> , Pb	25
	<b>Na<sub>2</sub>SO<sub>4</sub></b>	10
	CdS, Cd(ClO <sub>4</sub> ) <sub>2</sub> , ZnO, ZnSO <sub>4</sub> , FeS <sub>2</sub>	10

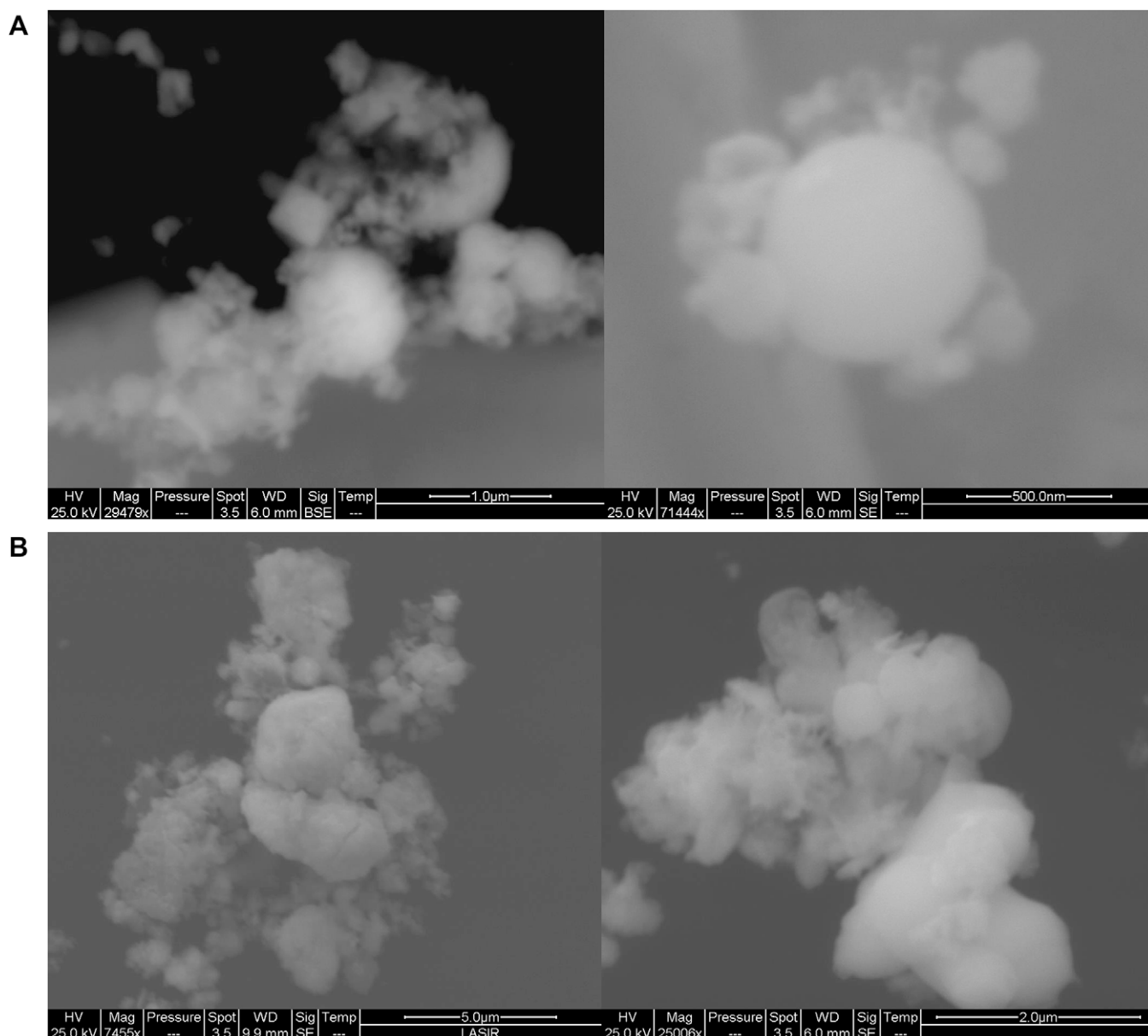
The major compounds are written in bold.

individual particles. The scanning of a large area of sample (300 μm<sup>2</sup>) by automated analyses enables the analysis of a huge number of particles. The data treatment of all the spectra recorded in this area leads to the major and minor component identification.

$x\text{PbO} \cdot \text{PbSO}_4$  ( $x = 1,2,3$ ),  $\text{PbSO}_4$ ,  $\alpha\text{-PbO}$ ,  $\text{PbCO}_3$ ,  $\text{Na}_2\text{SO}_4$ , and  $\text{ZnSO}_4$  were identified in both PM<sub>10</sub> and PM<sub>2.5</sub> samples as major species. The laser-damaging effect generates complete and irreversible oxidation of  $\text{PbS}$  to  $\text{PbSO}_4$  as described previously (Batonneau et al., 2000). The Raman mapping of species within particles exhibits a large heterogeneity of particle composition. A typical Raman image of PM<sub>2.5</sub> particles is shown in Fig. 3. A large number of particles are composed of lead sulphate and are agglomerated with other compounds such as sodium sulphate or carbonates in both PM<sub>10</sub> and PM<sub>2.5</sub> size fractions. The results suggest that no significant difference in lead speciation is observed in accordance with the particle size. However, for the minor lead compounds (like carbonates) not exactly quantified by XRD, differences in percentages between the two size fractions could exist.

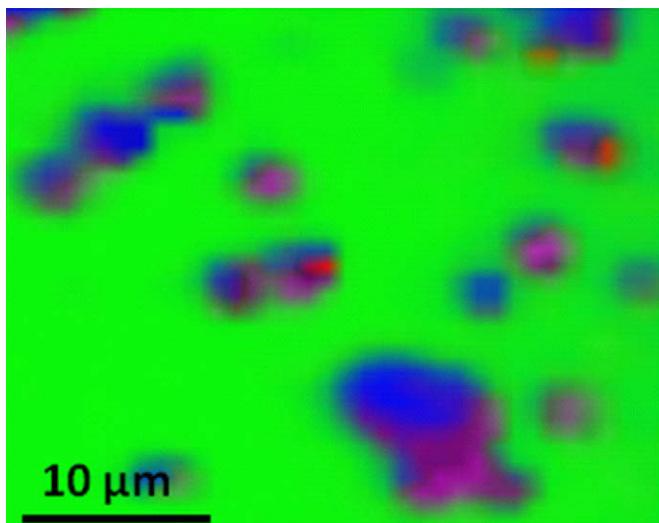
### 3.2. Assessment of the available fraction with $\text{CaCl}_2$ extraction

Fig. 4(A) and (B) shows respectively total lead concentrations extracted by  $\text{CaCl}_2$  and percentages of extracted lead with



**Fig. 2.** ESEM images of PM<sub>2.5</sub> (A) and PM<sub>10</sub> (B).





**Fig. 3.** Raman image of PM<sub>2.5</sub> particles after a smoothing procedure. Blue 4PbO·PbSO<sub>4</sub>, pink PbO·PbSO<sub>4</sub>, red Na<sub>2</sub>SO<sub>4</sub> and green substrate.

CaCl<sub>2</sub> (relative, with respect to total lead concentrations in different substrates) for various samples: the single particles, soil-1 and spiked soil-1 before and after an eight-day exposure of *L. sativa*.

Due to high levels of lead in particles (330,000 mg kg<sup>-1</sup>), maximum lead quantities were extracted from PM<sub>10</sub> and PM<sub>2.5</sub>. But, in comparison with total lead contents, lead in uncultivated spiked

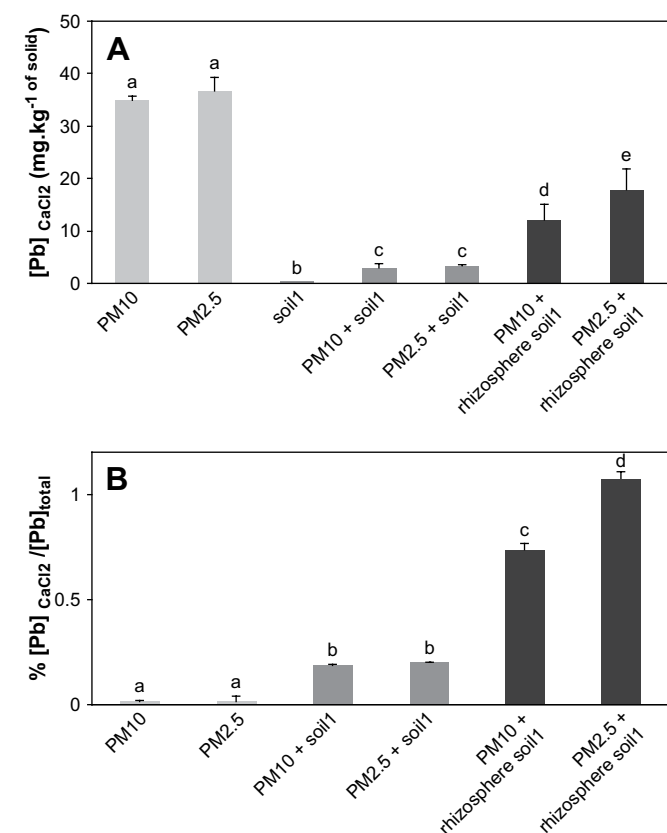
soils was 0.2% exchangeable, while PM<sub>10-2.5</sub> presented a 0.01% exchangeability. Reference soils were under ICP-OES limit detection. After culture, a significantly greater lead quantity was extracted by CaCl<sub>2</sub> for the soil spiked with the finest PM<sub>2.5</sub> fraction than in PM<sub>10</sub>. A one-unit pH decrease was measured after plant–soil contact while the pH values of the control soils (left without crops but with nutrient solution influence) remained constant. Concerning CaCl<sub>2</sub> extraction, no significant differences were observed between the two particle sizes in the PM<sub>10-2.5</sub> and spiked soil-1 before culture. The same trends described above for soil-1 were also observed for soil-2.

### 3.3. Influence of lead exposure on plant growth

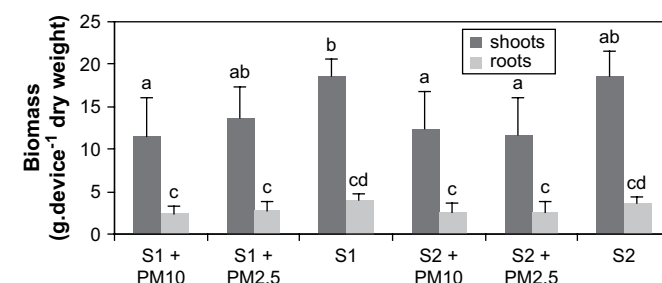
Fresh biomass data are shown in Fig. 5. After eight days of soil–plant exposure, biomasses for aerial parts grown on spiked soils were slightly lower than in the respective unspiked soils. No significant differences were observed between the two types of spiking (PM<sub>10-2.5</sub>). In controls, fresh weights were approximately 18.5 ± 2.5 g for aerial parts and 3.7 ± 0.7 g for roots while in spiked soils, weights reached 12 ± 4 g and 2.5 ± 1 g respectively. This slight influence of lead on plant biomass could be due to lead toxicity (Sharma and Dubey, 2005) or water status (Parys et al., 1998).

### 3.4. Absorption and adsorption of lead in the lettuce

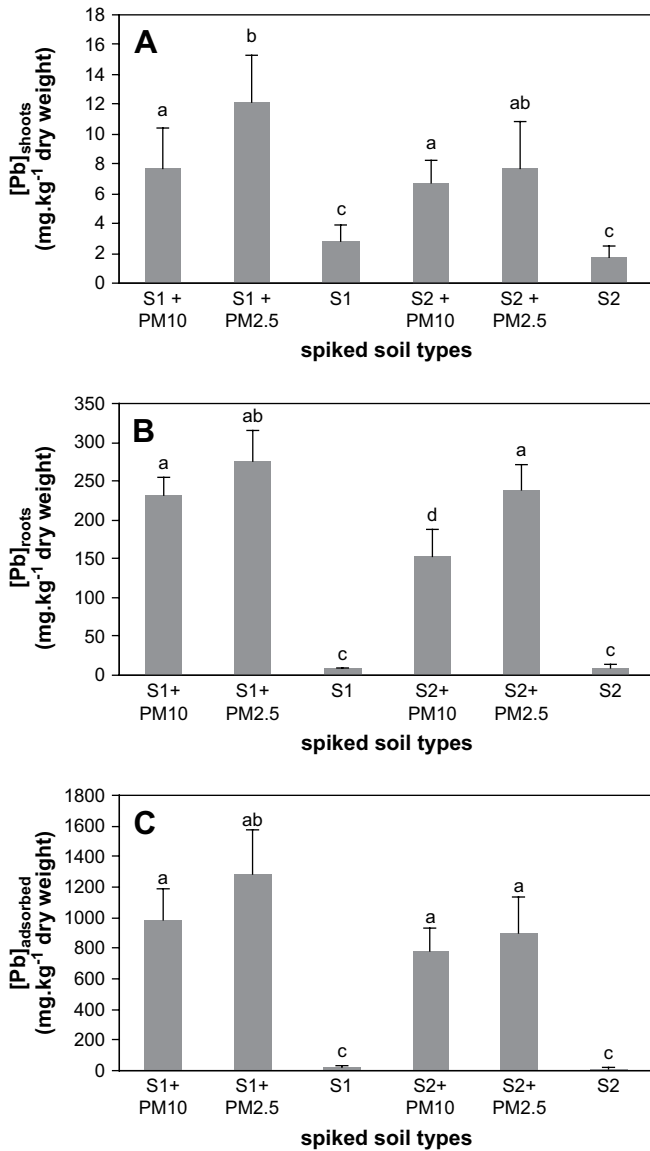
Fig. 6 presents results of lead transfer from soil to the various compartments of the plant (roots and shoots) and the distribution between adsorption (noted [Pb]<sub>adsorbed</sub>) and absorption for roots ([Pb]<sub>roots</sub>) respectively described in Section 2 as lead only adsorbed at the root surface and lead truly taken up by the plant. Measurements on the reference samples (Tobacco leaf VTL-2) validated the assay: the concentration found was 21.4 ± 1.1 mg kg<sup>-1</sup>, for a certified value of 22.1 ± 1.2 mg kg<sup>-1</sup>. Several trends were observed dealing with the influence of the particle type, soil type and lead location. For both soils, a significant increase of lead adsorption on roots, absorption and translocation throughout the shoots was observed, when the finest particles were added in comparison with the PM<sub>10</sub>. Global uptakes ([Pb]<sub>adsorbed</sub> + [Pb]<sub>roots</sub> + [Pb]<sub>shoots</sub>) were greater for soil-1 than for soil-2. The main lead fraction was adsorbed on root membranes where concentrations were up to 985 ± 147 mg kg<sup>-1</sup> for PM<sub>10</sub> contaminated soils and up to 1281 ± 195 mg kg<sup>-1</sup> for PM<sub>2.5</sub> contaminated soils. The soils spiked with PM<sub>10</sub> allowed a lead uptake of 230 ± 24 mg kg<sup>-1</sup> by roots and a translocation to aerial parts of 7.7 ± 2.7 mg kg<sup>-1</sup>. While in the PM<sub>2.5</sub> spiked soils, root and shoot concentrations reached 275 ± 40 mg kg<sup>-1</sup> and 12.19 ± 3 mg kg<sup>-1</sup> respectively.



**Fig. 4.** Total contents (A) and relative contents (B) of lead extracted with CaCl<sub>2</sub> on particles (white), spiked soils with lead particles of different size before (grey) and after (black) a seven days exposure of *Lactuca sativa*, n = 5.



**Fig. 5.** Plant biomass data for aerial parts (black) and roots (grey). The error bars indicate the standard deviation while different letters show significant differences ( $p < 0.05$ ) as measured by an LSD Fisher test (one factor ANOVA).



**Fig. 6.** Lead concentrations in different plant compartments: (A), adsorbed onto roots; (B), root uptake; and (C), translocated in shoots.

## 4. Discussion

### 4.1. Influence of particle size on soil–plant transfer and lead translocation

Whatever the soil, for a given total lead concentration (1650 mg Pb kg<sup>-1</sup> soil), higher lead soil–plant transfer and translocation were observed for the finest particles. Roots exposed to PM<sub>2.5</sub> spiked soils allowed a 20% greater lead uptake and a 30% increase in adsorbed lead. Shoots presented a 60% increase in translocated lead in PM<sub>10</sub> spiked soils. For the first time in industrial particles, the transfer of lead to the soil solution and its translocation throughout the plant is reported to increase as the particle size decreases.

Douay et al. (2005) measuring lead concentrations in lettuces in urban gardens (1572 mg Pb kg<sup>-1</sup>) observed a shoot concentration of 5 mg Pb kg<sup>-1</sup> DW. The soil to plant metal transfer measured in this study (for one week of lead exposure) was relatively high in comparison with previously reported data in publications dealing with vegetables (BAPPET, 2008), probably not only because of particle size, but also due to numerous factors that influence the

soil–plant transfer of metals (Kabata-Pendias, 2004; Dumat et al., 2001, 2006; Costa and Morel, 1993; Oliver et al., 1994).

Root transfer factors (TF-roots) and shoot transfer factors (TF-shoots) were calculated as ratios between [Pb]<sub>roots</sub>, [Pb]<sub>shoots</sub> and [Pb]<sub>soil</sub>. TFr ranged from 0.10 to 0.17 and TFs were between 0.005 and 0.007 (Table 4). These relatively low values illustrate the known low mobility and availability of lead and are comparable to Khan et al.'s results (2008) reporting 0.15 for TFr and 0.07 for TFs. As transfer factors for vegetables decrease with increasing levels of lead in the soil (Zheng et al., 2007; Wang et al., 2006), we therefore expected a greater influence of particle size on transfer with a lower total lead concentration in soil.

According to Sobanska et al. (1999), lead smelter emissions contain PbS, PbSO<sub>4</sub>, PbSO<sub>4</sub>·PbO, Pb, α-PbO compounds and numerous nanoballs were observed for PM<sub>2.5</sub>. Dumat et al. (2001) and Cecchi et al. (2008) working on soils polluted by lead produced by industrial atmospheric fallout from smelters, concluded that lead chemical speciation strongly influences its bioavailability. The sequence of solubility constants from the CHESS database for the lead is of the following: PbCO<sub>3</sub> > PbSO<sub>4</sub> > PbO > Pb<sup>0</sup>. In water media and equilibrium conditions, lead carbonate and sulphate will be much more labile than PbO or Pb<sup>0</sup> species. However, according to Birkefeld et al. (2006, 2007) which used an *in situ* method to study dissolution and phase transformation of lead particles from a smelter in different soils, the sequence of solubility is strongly dependent on soil characteristics (like texture, pH, lime amount, etc.). They observed that PbO was rapidly covered by lead-hydroxy carbonates (hydrocerussite) in some soils while in other it was relatively stable. Moreover, according to the general review of Ruby et al. (1992), release of lead in the soil solution depends on particle size, speciation and soil geochemistry. In soil solution, ligands like fulvic acids or low weight organic acids excreted by roots can displace the equilibrium (Ferrand et al., 2006). Finally, as no significant difference among speciation forms was noticed between PM<sub>10</sub> and PM<sub>2.5</sub>, all changes observed in reactivity were attributed to size differences.

Whatever the particle size, greater lead absorption by lettuce was observed for soil-1 than for soil-2. In comparison with soil-2, soil-1 has more clay, its CEC is higher and its level of lime is lower (see Table 1). According to Twining et al. (2004), we could expect that lead transfer would be higher for soil-2. However, several hypotheses can explain the results observed: (i) the higher amount of carbonates present in the soil-2 could reduce lead absorption (Birkefeld et al., 2006, 2007); (ii) the complex influence of soil organic matter on the transfer of metals (Yin et al., 2002; Inaba and Takenaka, 2005); (iii) for the total lead concentration studied, the relatively high quantity of lead could be available in the soil solution (due to high solubility of the fine particles) reducing the influence of soil characteristics.

Total lead in the roots ([Pb]<sub>roots</sub> + [Pb]<sub>adsorbed</sub>) reached 1500 mg Pb kg<sup>-1</sup> dry weight. Lead in roots was predominantly adsorbed onto the cell walls, 80% of the total lead in roots, and depends on the nature of the particles used for exposure: the uptake was greater for PM<sub>2.5</sub> than PM<sub>10</sub>. According to Seregin and Kozhevnikova (2004), Pb<sup>2+</sup> binds to the carboxy groups at the root surface reducing the translocation rate of lead (Pendergrass and Butcher, 2006;

**Table 4**

Transfer factors in roots (TFr) and shoots (TFs) and translocation ratios

Soil	TFs	TFr	Translocation
S1 + PM <sub>10</sub>	0.005	0.14	0.03
S1 + PM <sub>2.5</sub>	0.007	0.17	0.04
S1	0.081	0.26	0.31
S2 + PM <sub>10</sub>	0.004	0.10	0.04
S2 + PM <sub>2.5</sub>	0.005	0.15	0.03
S2	0.01	0.44	0.18

Piechalak et al., 2002). To illustrate this phenomenon, the translocation factors or shoot/root ratios calculated indicate the ability of plants to transport metals from the roots towards the aerial parts (Ferrand et al., 2006): they ranged between 0.03 and 0.04 (Table 4). But considering the lower biomasses in roots than in shoots, we can also reason with lead quantities. Whatever the type of soil or spiking, the total lead quantity in shoots ( $Pb_{shoots} \times \text{dry weight}$ ) between 0.003 and 0.007 mg Pb translocated which represents up to 33% ( $PM_{2.5}$ ) of the total lead uptake. This percentage is not negligible with respect to risks concerning consumption of vegetables.

#### 4.2. Behaviour of lead in the rhizosphere and assessment of lead availability

Relatively high lead transfer was observed for the lettuce cultivated on the two alkaline spiked calcareous soils.  $PM_{10-2.5}$  can therefore release lead in particular in the rhizosphere of *L. sativa*. The lead availability estimated by the  $CaCl_2$  procedure was greater for soils spiked with the finest particles and for both soils it increased after soil-plant contact:  $[Pb]_{CaCl_2}$  in spiked soil with  $PM_{2.5} > [Pb]_{CaCl_2}$  in spiked soil with  $PM_{10}$ . Under the root activity influence, the  $CaCl_2$  lead extracted from polluted soils increased and a one-unit pH decrease in soil was measured. Lin et al. (2004) and Kidd and Monterroso (2005) also observed that exchangeable lead was much higher in the rhizosphere than in the bulk soil. Producing exudates, plants can modify metal speciation and behaviour in the rhizosphere (Lin et al., 2004; Laperche et al., 1997; Welch, 1995). This phenomenon has been particularly observed for calcareous soil by Chaignon and Hinsinger (2003). As pH influences metal solubility and transfer (Wang et al., 2006), the rhizosphere acidification could have displaced the equilibrium towards bicarbonates, which are less stable than carbonates (Sauvé et al., 1998). An effect of soil on particle solubility was also observed: particles present a  $CaCl_2$  exchangeability ten times lower than in spiked soil. Quantities extracted for particles were only up to 1.5% of the solution extraction. Mixed with soil for four months, particle solubility could therefore have changed.

In order to estimate the transfer of lead from polluted soils towards lettuce, relationships were sought between: lead mobilized by  $CaCl_2$  extraction performed on soils before culture and lead concentrations in lettuce (shoots and roots). Equations were obtained from 6 parameters (2 soils; uncontaminated, spiked with  $PM_{10}$  or  $PM_{2.5}$ ), and every condition was studied by 5 replicates finally involving 30 observations. Significant correlations were observed between lead concentrations in shoots (Eq. (1), with  $r^2 = 0.8$ ) or in roots (Eq. (2), with  $r^2 = 0.9$ ) and lead extracted by  $CaCl_2$ .

$$[Pb]_{shoots} = 2.11 \times [Pb]_{CaCl_2} + 2.13, \quad r^2 = 0.797, \quad p < 0.01, \quad n = 30 \quad (1)$$

$$[Pb]_{roots} = 35.02 \times [Pb]_{CaCl_2} + 4.24, \quad r^2 = 0.915, \quad p < 0.005, \quad n = 30 \quad (2)$$

Both for  $PM_{10}$  and  $PM_{2.5}$ , the  $CaCl_2$  chemical extraction was therefore a good indicator of soil-plant transfer. As previously shown by Pueyo et al. (2004), the  $0.01 \text{ mol L}^{-1}$   $CaCl_2$  extraction procedure seems to be a suitable method for the determination of phytoavailable lead. This test simplifies the matrix and could avoid *in vivo* tests.

## 5. Conclusions and perspectives

A significant size influence was found for soil-plant lead transfer and translocation throughout the lettuce when micronic and

nanometric industrial particles were compared: roots exposed to  $PM_{2.5}$  spiked soils allowed a 20% greater lead uptake and a 30% increase in adsorbed lead. Shoots presented a 60% increase in translocated lead in  $PM_{10}$  spiked soils. Our results highlight that source characteristics strongly influence metal transfer: total metal soil concentration is insufficient to estimate the risk induced by soils polluted by metals. Lead speciation was quite similar in  $PM_{10}$  and  $PM_{2.5}$  particles, i.e.  $PbS$ ,  $PbO \cdot PbSO_4$ ,  $PbO$ ,  $PbCO_3$  and  $Pb^0$ , were predominant however differences could exist for minor lead components. Moreover, the high amount of Pb-rich nanoballs and nanocrystals much more dispersed for  $PM_{2.5}$  could influence the lead transfer in the rhizosphere acidified by plant root activity. Lead concentrations in the edible part of lettuces were very well estimated by 0.01 M  $CaCl_2$  extraction.

In order to check the generality of the size influence of industrial particles on soil-plant transfer, further experiments could be performed on other vegetables and various soils testing aging effects. Moreover, the study of other metals and metalloids measured in the particles will be performed.

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